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The Impact of Midbrain Cauterize Size on Auditory and Visual Responses' Distribution

Yan Zhang

THE IMPACT OF MIDBRAIN CAUTERIZE SIZE ON AUDITORY AND VISUAL RESPONSES' DISTRIBUTION

by

YAN ZHANG

Under the Direction of Dr. Yu-Sheng Hsu

ABSTRACT

This thesis presents several statistical analyses on a cooperative project with Dr. Pallas and Yuting Mao from Biology Department of Georgia State University. This research concludes the impact of cauterize size of animals' midbrain on auditory and visual response in brains. Besides some already commonly used statistical analysis method, such as MANOVA and Frequency Test, a unique combination of Permutation Test, Kolmogorov-Smirnov Test and Wilcoxon Rank Sum Test is applied to our non-parametric data. Some simulation results show the Permutation Test we used has very good powers, and fits the need for this study. The result confirms part of the Biology Department's hypothesis statistically and enhances more complete understanding of the experiments and the potential impact of helping patients with Acquired Brain Injury.

INDEX WORDS: MANOVA, Frequency Test, Kolmogorov-Smirnov Test, Wilcoxon Rank Sum Test, Pearson Chi-square Test, Fisher's Exact Test, Permutation Test

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LIST OF ABBREVIATIONS

ABI	Acquired Brain Injury
AI	Primary Auditory Cortex
MANOVA	Multivariate Analysis of Variance
KS-Test	Kolmogorov-Smirnov Test
MRI	Magnetic Resonance Imaging

CHAPTER ONE: INTRODUCTION

1.1 Background

Acquired Brain Injury (ABI) is a serious clinical problem. It can be caused by traumatic brain injury (physical trauma) and non-traumatic brain injury (stroke, brain tumor, etc.). ABI can affect cognitive, physical, emotional, social and any other functions in the brain. The outcomes of ABI range from complete recovery to death or permanent disability, largely depending on the severity of the injury and timely receipt of appropriate treatment. The demand to save lives of soldiers from traumatic brain damage is increasing because of the increased incidence of closed head, blast-induced injury in war zones. The knowledge from research on ABI will not only help to understand the mechanisms that underlie ABI, but may also be applied to improve medical treatment of patients with ABI.

Research on ABI has primarily concentrated on recovery from injury and compensatory plasticity. Compared to other models such as congenitally deaf animals, the cross-modal plasticity model initialized by Dr. Sarah Pallas and her assistant Yuting Mao is especially suitable for studying ABI, taking the advantage of neonatal injury in brain areas. In their model system, midbrain lesions cause visual afferents to project to auditory thalamus. This process is an example of the process of recovery from brain injury. The primary auditory cortex is then reorganized across modalities, providing an example of compensatory plasticity. This model offers an opportunity to several future research topics on ABI. We only helped analyzing the auditory and visual responses' distribution on the primary auditory cortex after the ABI using appropriate statistical methods.

The cross-modal plasticity model initialized by Dr. Sarah Pallas and Yuting Mao uses ferrets as the experimental subjects. They cauterized midbrain of ferrets at postnatal day one. Each animal was scanned by Magnetic Resonance Imaging (MRI) to confirm the midbrain lesions. In vivo extracellular recording was performed in adulthood. Auditory, visual and bimodal stimuli were applied during recording. Electrical stimulation of the optic chiasm was used to activate optic nerves directly in the event that there was no response to light. Normal animals without any surgery were used as a control for the lesion group.

The topic of this thesis is about analyzing the impact of midbrain lesion size on the distribution of the response points based on their modality and whether the visual response is different from the auditory response and multimodal response in position on the primary auditory cortex.

1.2 Source of Data

Data used in this thesis is provided by Dr. Sarah Pallas and her graduate assistant Yuting Mao from Biology Department of Georgia State University. They started the experiments since 2007.

There are 3 lesion groups- Control, Small lesion and Large lesion. Control group has 9 animals. Small lesion group has 8 animals. Large lesion group has 3 animals. In total we have 775 pairs of two dimensional data. There are no missing values.

The experimenters found the positions of neurons on the primary auditory cortex that have responses to either visual or auditory stimuli or both. Neurons that only respond to visual stimuli were defined as visual neuron (red dots). Neurons that only respond to auditory stimuli were defined as auditory neuron (blue dots). Neurons that respond to both auditory and visual

stimuli were defined as multimodal neurons (dots that have both blue and red colors). Neurons that only respond to one modality and can be significantly modulated by another modality were defined as multimodal neurons (dots that have both blue and red colors). The first version of the data and the illustrations of the animals' brain indicated multimodal neurons with green color. Only at the final step of our analysis, Yuting Mao updated their data using dots of both blue and red color for multimodal neurons. We could see that from updated Figure2, Figure 3 and Figure 4. But our code and some preparation work and graphs already used green color for multimodal neurons. We will still use green color to indicate multimodal neurons through this paper for our convenience.

Upon Dr. Yu-sheng Hsu's request, the position of neurons was standardized into X-Y Cartesian plot. Y-axis is the distance from the tip of field anterior ectosylvian sulcus (fAES) to the top of ectosylvian gyrus. X-axis is the axis that is perpendicular to y-axis (Fig 1). The size of auditor cortex from all animals was standardized when the length of Y-axis equals to one.

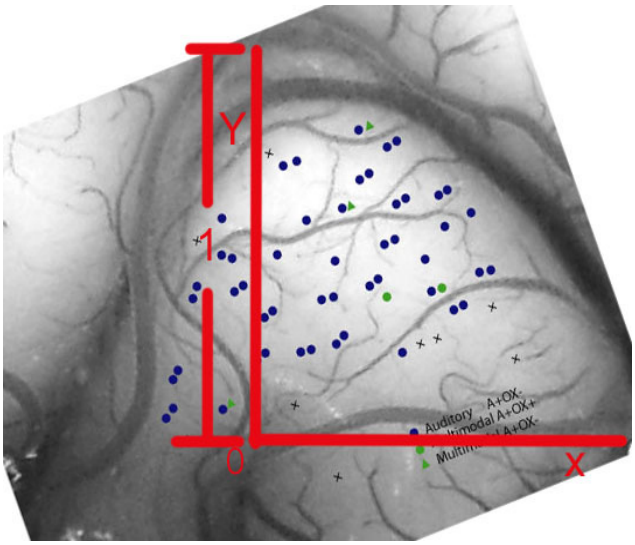


Figure 1 Cartesian plot on AI

The following figures were provided by Yuting Mao along with the measured *x* and *y* values.

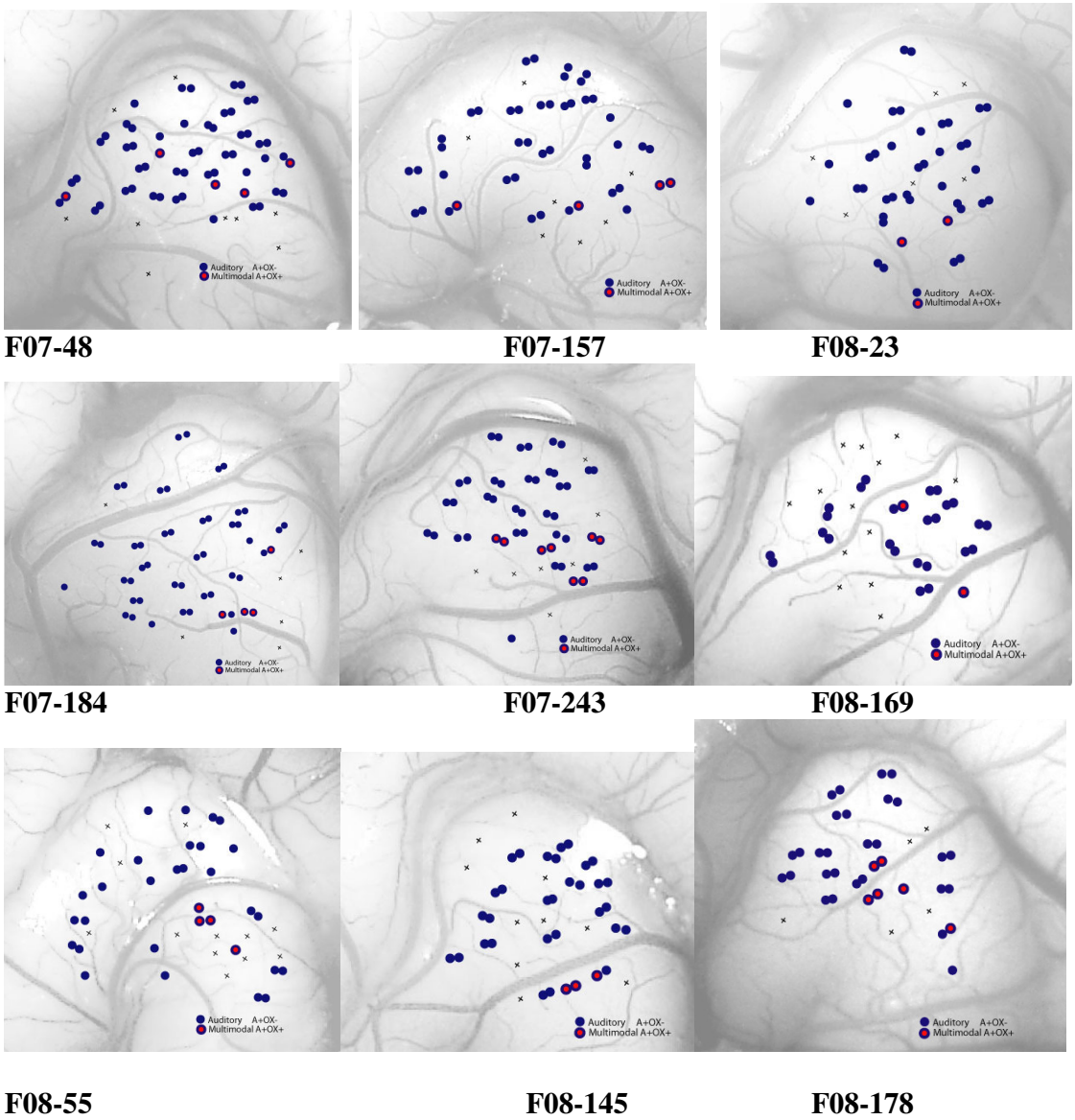


Figure 2 Control group (blue dots—auditory neurons; mixed color dots—multimodal neurons)

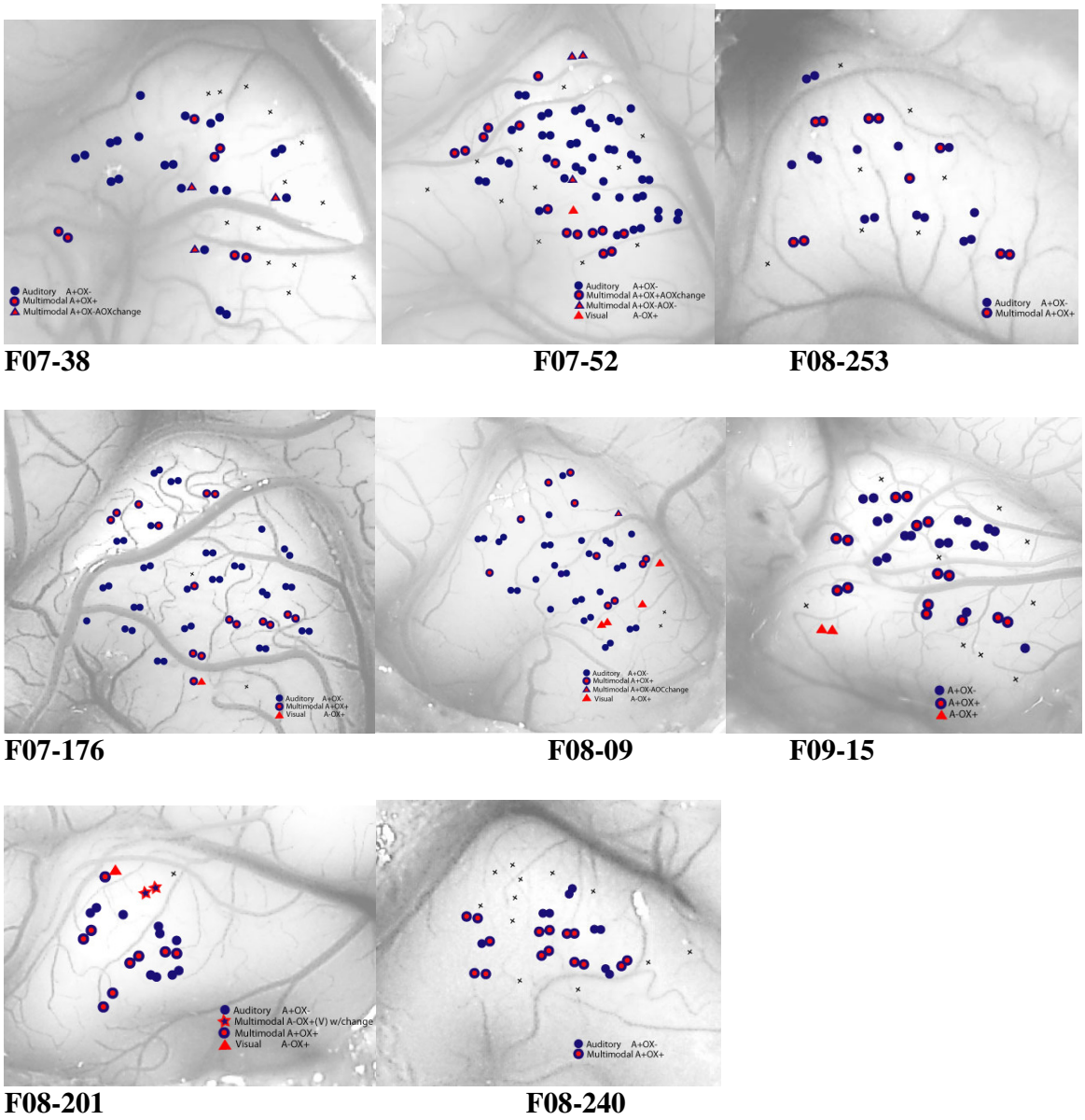
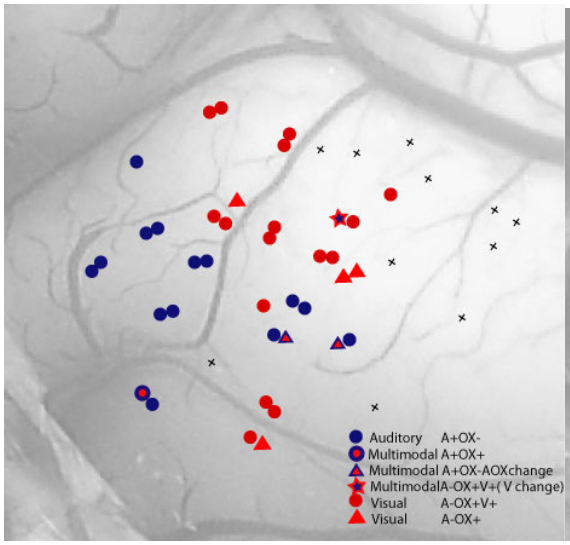


Figure 3 Small lesion group (blue dots--auditory neurons; mixed color dots-multimodal neurons; red dots--visual neurons)



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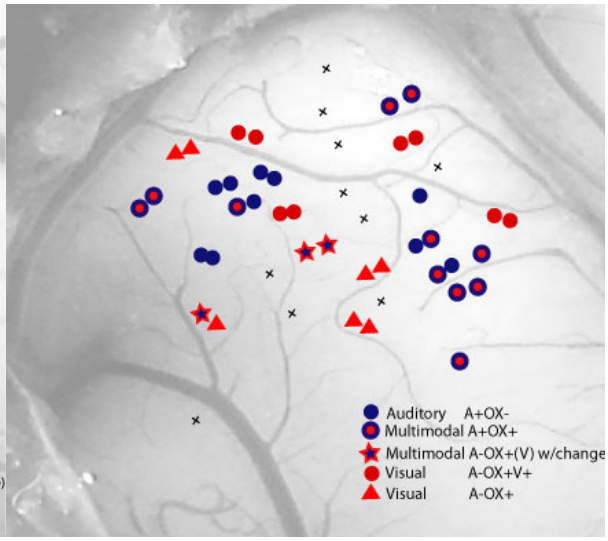

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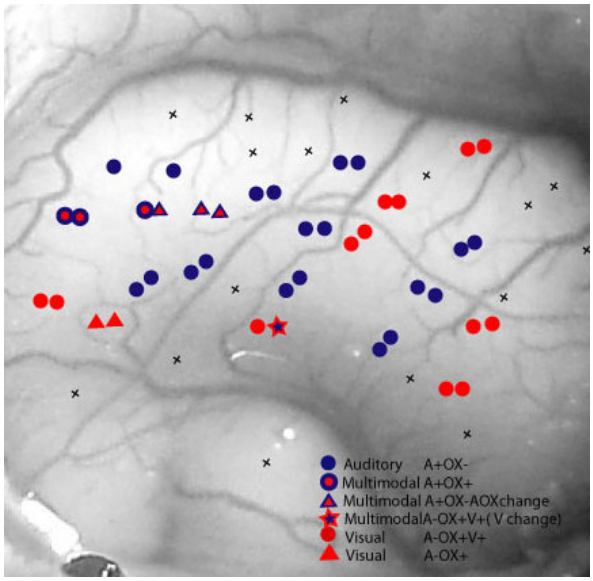

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Figure 4 Large lesion group (blue dots--auditory neurons; mixed color dots-multimodal neurons; red dots--visual neurons)

CHAPTER TWO: METHOD OF ANALYSIS

In this chapter, we discuss and review the techniques elected to analyze the provided data and then draw the conclusion. We first did a Frequency Test to get a general idea of the data. On our second step, to test the impact of three selected potential factors on the location of those response dots (in terms of x and y), we applied MANOVA test on all the data as a whole and then within each lesion group. Last, under Dr Hsu's guidance, we used a unique combination of Kolmogorov Smirnov test, Wilcoxon Rank Sum Test and Permutation Test on the two dimensional data to detect any distribution difference on same color points from different lesion groups.

2.1 Frequency Test

Frequency Test is very commonly used in statistics analysis. In our analysis, we are interested in the frequency test on two classification cross table since we want to know if there is any association between factor color and factor lesion size. In this case the null hypothesis is that the two factors are independent.

From the contingency table of factor color and factor lesion size, we could already see the change of the percentage of each color within each lesion size group. Furthermore, we requested both Pearson Chi-square Test and Fisher's Exact Test through SAS code to obtain the P-value. Fisher's Exact Probability is requested because Fisher's Exact Probability is not plagued by inaccuracies due to small number of observations. We noticed that some animals have only one or two observations for certain colors.

2.2 Multivariate Analysis of Variance (MANOVA) Test

In our analysis, we are interested in knowing if the location of response points is affected by the following three factors: Which animal do these points belong to? What color are these points? And what kind of lesion was this animal given? Since the location is indicated by x and y, MANOVA Test answers these questions by testing the hypothesis on the means of (x, y) for each group of points applying these three independent factors----lesion size, color and individual animal. The factor, individual animal, is nested within factor lesion size.

2.3 Permutation Test

The last topic of interest is to detect that whether points of the same color between different lesion groups have same distributions. This part of analysis was initialed by Dr. Hsu using a unique combination of three non-parametric methods and is the emphasis of this thesis. It was programmed by us because there are no available functions ready to use in SAS. I'd like to introduce our methodology in details about this part.

The most common methods for inference about means based on a single sample, matched pairs, or two independent samples (our case) are the t procedures. We always rely on the assumption of normal distribution for data but no data are exactly normal. The t procedures are robust, quite insensitive to deviations from normality in the data. But we usually need quite large samples. The datasets we were given are typical biology experiment outcomes. We have small number of objects---9 Control group animals, 8 Small lesion animals and 3 Large lesion animals. Besides the limited number of observations, these three groups of observations are not balanced. Obviously, it is not appropriate to use traditional inference on our limited datasets. We used Permutation Test because it keeps the computing power but relax some of the conditions needed

for traditional inference. We don't know what kind of distribution those response points have and each animal's response to the lesion surgery is quite individual. Permutation Test sets us free from the need for normal data or large samples and it also set us free from formulas or distributions. It also works well for unbalance data like ours. Because of its effectiveness and range of great use, Permutation Test along with other randomization test is becoming the preferred way to do statistical inference. It is already true in some areas such as clinical trial and bio-medical research. It can, with sufficient computing power, gives results that are more accurate than those from traditional methods. So before we applied the permutation test on the real data, we first did the simulation to check the power of the test. The results are actually better than we expected. Please refer to the simulation table in the discussion chapter.

We first decided that the test statistics to detect the distribution difference is a measure of "distance" between two multivariate distributions. We selected Kolmogorov-Smirnov Distance. The available K-S Test in SAS is only for one dimension data so we tailored our own code for our two dimensional data. Then we did a transfer of our test statistics from the solid number of "distance" out of any two animals to its corresponding rank then to the Rank Sum of all the animals in one lesion size population using Wilcoxon Rank Sum Test. After that we used Permutation Test to list all the possible rank sums from all possible ways of relocating animals to two groups and obtained the significance test result. We cannot use the approximate distribution of the Wilcoxon Rank Sum statistic because the "independent" condition is not satisfied in this Permutation Test.

2.3.1. Kolmogorov-Smirnov Test

First, we selected a measure of distance between two multivariate distributions. We used the two-sample Kolmogorov-Smirnov Test (KS-test). It tries to determine if two distributions differ significantly, i.e., to test whether two samples come from the same distributions. The KS-test has the advantage of making no assumption about the distribution of data. Technically speaking it is non-parametric and distribution free.

We have animal #1 with *n1* response points and animal #2 with *n2* response points. When you mix these two animals' response points, for each dot of (*x, y*) from the mixed pool, you will find F_{n1} and F_{n2}, where $F_n(x, y) = \frac{1}{n}\sum_{i=1}^{n} I_{(X,Y)_i \le (x,y)}$. Use the following formula, you will find a *D* given any two animals' response points.

$$D_{n1,n2} = \sup_{(x,y)} \left| F_{n1}(x, y) - F_{n2}(x, y) \right|$$

If the two animals' same colored points are distributed at approximately same location, their cumulative fraction plots should be close to each other, producing small value of *D* statistic. If the two animals' same colored points are distributed far away from each other, their data will give us large value of *D* statistic.

Suppose we have M animals from lesion group 1 and N animals from lesion group 2. Since we can work out a *D* statistic for any two animals, we will have $\binom{M}{2}$ *D*s if both animals are from lesion group 1 and $\binom{N}{2}$ *D*s if both animals are from lesion group 2. There are M*N *D*s if one animal from each lesion group. We will see this equation:

$$\binom{M}{2} + \binom{N}{2} + M * N = \binom{M+N}{2}$$

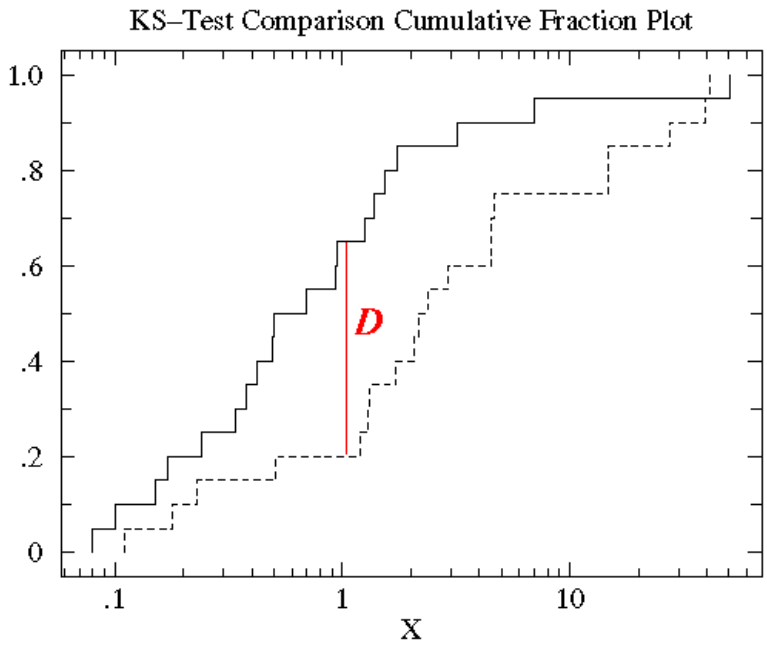

Figure 5 Illustration of KS-test's *D* statistic

2.3.2. Wilcoxon Rank Sum Test

The Wilcoxon Rank Sum Test can be used to test the null hypothesis that two independent samples of observations X and Y come from the same distribution. It is one of the best-known non-parametric significance tests that do not require assumptions about the form of the distribution of the measurements. It is very efficient for small sample size like our case.

Assume we have sample of size m from population X and sample of size n from population Y. We then merge the data and rank each component from lowest to highest. All sequences of ties are assigned an average rank. The Wilcoxon test statistic *W* is the sum of the ranks from population X or Y. Unusual small or large W indicates the rejection of the null hypothesis of no difference in two distributions.

Now Wilcoxon Rank Sum Test is applied on all the $\binom{M+N}{2}$ D statistics worked out from step I. We consider $\binom{M}{2}+\binom{N}{2}$ Ds are from population X, which means both animals are from the same lesion group and the Ds are relatively small, so the assigned ranks on them are also small. While $M * N$ Ds are from population Y, which means two animals are from different lesion group and the Ds are relatively large, therefore the assigned ranks on them are also large.

If we use the rank sum of population X, small statistic W would lead us to reject null hypothesis of no distribution difference of same colored points from different lesion groups. For the convenience of our program writing, we used the rank sum of population Y as the Wilcoxon Test statistics. Therefore, large number result in our program code leads to the rejection of null hypothesis.

2.3.3. Permutation Test

Now with the test statistic W from the above Wilcoxon Rank Sum step, we will do the statistical significance test to see if we can reject null hypothesis or not. Since we don't know either the distribution of the points or the distribution of Wilcoxon Rank Sum statistic from last step due to the dependency of X and Y, we chose to do the Permutation Test.

Permutation test (also called a randomization test) has the advantage of being a non-parametric statistics test. It is a type of statistical significance test in which a reference distribution is obtained by calculating all possible values of the test statistics under rearrangements of the labels on the observed data points. If the labels are exchangeable under the null hypothesis, then the resulting tests yield exact significance levels.

An important assumption behind a Permutation Test is that the observations are exchangeable under the null hypothesis. Our project has this assumption to start Permutation Test with. Under the null hypothesis, we assume the same colored points from different lesion groups come from the same distribution. Suppose we have two groups *A* and *B*. Let n_A and n_B be the sample size corresponding to each group. The permutation test is designed to determine whether the Wilcoxon Rank Sum of population Y (two animals are from different lesion groups) is large enough to reject the null hypothesis H_0 that the two groups' same colored points have same distribution. The test proceeds as follows. First, the actual Rank Sum *W* is calculated: this is the observed value of the test statistic. Then the observations of groups *A* and *B* are pooled. Next, the Rank Sum *W* is calculated and recorded for every possible way of dividing these pooled values into two groups of size n_A and n_B (i.e., for every permutation of the group labels A and B). The set of these calculated rank sum *W*s is the exact distribution of all possible test statistic *W*s under the null hypothesis that group label does not matter. In our test, we sorted the entire recorded test statistic *W*s, and then see if the observed value of *W* from first step is contained within the lower 95% of them. If the observed *W* belongs to the upper 5%, we reject the null hypothesis of identical distribution for same colored points from different lesion groups at the 5% significant level.

Before we did this test, we did simulation to see the power of this Permutation Test combined with Kolmogorov-Smirnov Test and Wilcoxon Rank Sum Test. We will discuss the result in our next chapter.

CHAPTER THREE: RESULTS AND DISCUSSIONS

Now, let's look at the analysis results when we apply the techniques mentioned in Chapter two to the provided data.

3.1 Frequency Test Result

A graph was provided by Yuting Mao from the Biology Department before we started our statistical analysis. It visually shows the percentage composition of blue, green and red points within each lesion group. Later we updated this graph using all the datasets provided.

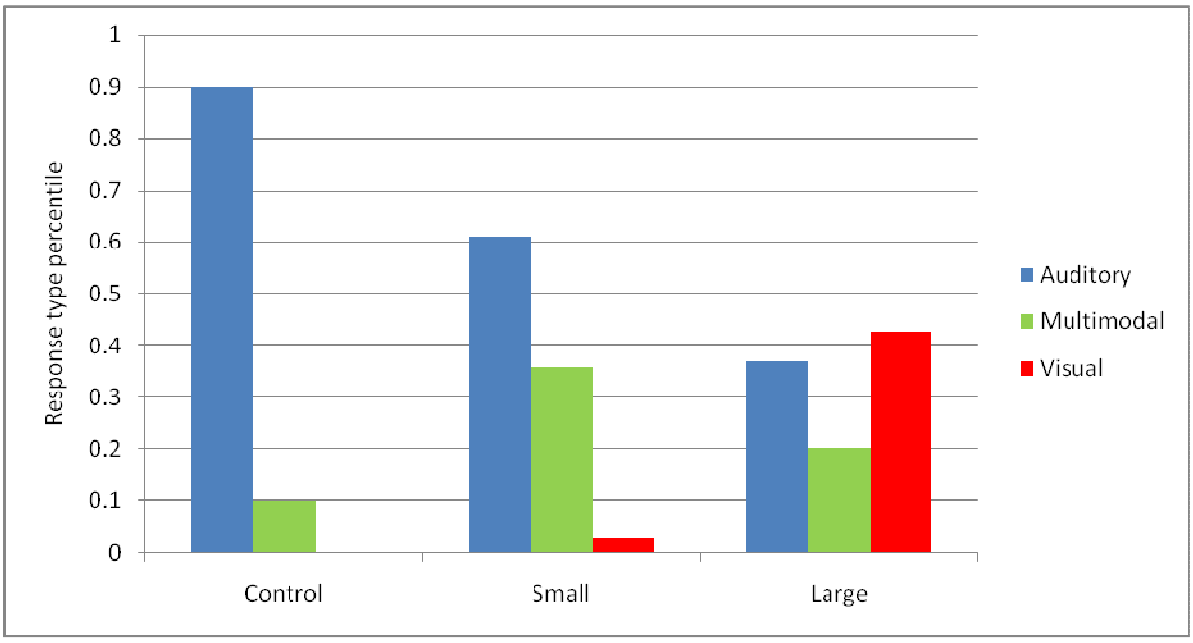

Figure 6 Proportion compositions (blue dots for Auditory, green dots for Multimodal and red dots for Visual responses)

Visually we can see that for Control group animals, there are no red (visual response) points at all. With the change of lesion size from Control to Small to Large, the percentage composition of blue (auditory response) points decreases, while the percentage composition of red (visual response) points increase, suppressing the other two types of neurons. The trend for

multimodal responses points is not obvious from Small lesion group to Large lesion group. Do our data also tell us the trend of changes is affected by lesion size statistically?

The following Table 1 comes out of our SAS result. We could see the consistent changes with the above figure. Pearson's Chi-square Test shows a P-value of <0.0001. Fisher's Exact Test gives us a P-value of 2.812E-58. (Appendix: SAS result) With the extremely small P-values from both tests, we reject the null hypothesis that there is no association between lesion size and the color of the points.

Table 1 Percentage composition of each color group within each lesion group

Lesion Size	***Auditory***	***Multimodal***	***Visual***
Control	89.86%	10.14%	0
Small	61.13%	35.88%	2.99%
Large	36.97%	20.17%	42.86%

3.2 MANOVA Test to Show the Impact of Three Factors on the Location of the Response Points

We did the MANOVA Test twice, once on all the combined data to find the overall impact of our three factors and once within each lesion group. In our regression, the location of each point (indicated by x and y) is the response variable. There are three regressors: lesion size, color of that point and each individual animal. Each individual animal is nested within its lesion size.

For the overall dataset analysis, we created the following P-value table:

Table 2 MANOVA table of all data: Impact of three factors on *x*

Source	*DF*	*Type III SS*	*Mean Square*	*F Value*	*P-value*
lesion	2	0.25947762	0.12973881	2.46	0.0859
color	2	0.36347503	0.18173752	3.45	0.0323
id(lesion)	17	1.73084747	0.10181456	1.93	0.0131

Table 3 MANOVA table of all data: Impact of three factors on *y*

Source	*DF*	*Type III SS*	*Mean Square*	*F Value*	*P-value*
lesion	2	1.83761457	0.91880729	27.33	<0.0001
color	2	0.24688497	0.12344249	3.67	0.0259
id(lesion)	17	3.88071811	0.22827754	6.79	<0.0001

Table 4 P-value table of MANOVA on all data: Testing overall factor effect

Statistic	*No lesion Effect*	*No color Effect*	*No id(lesion) Effect*
Wilks' Lambda	<0.0001	0.0084	<0.0001
Pillai's Trace	<0.0001	0.0085	<0.0001
Hotelling-Lawley Trace	<0.0001	0.0084	<0.0001
Roy's Greatest Root	<0.0001	0.0024	<0.0001

Based on the above tables' result, we conclude that:

1. Under 5% significant level, lesion size is not a very significant factor on *x* value since the P-value of the impact of lesion size on *x* is 0.0859. The other two factors are both significant factor for *x* value. All three factors are significant to predict *y*

value. *Y* value is more sensitive to the impact of all three factors than *x* value. (result from Table 2 and Table 3)

2. When testing the null hypothesis of No Overall lesion Effect on the location of points, we found that all the available statistics lead to strong rejection of null hypothesis.
3. When testing the null hypothesis of No Overall color Effect on the location of points, we found that all the available statistics lead to strong rejection of null hypothesis.
4. When testing the null hypothesis of No Overall individual animal Effect on the location of points, we found that all the available statistics lead to strong rejection of null hypothesis. (Conclusion 2, 3 and 4 are from Table 4)

The above result shows that all three factors play significant roles in determining the location of each point.

Then we did the MANOVA Test within each lesion group. Within each lesion group, we only tested the factor color and individual animal. We had the following P-value tables and conclusions:

1. Within the Control group, factor color is still a significant factor on the location of a point. This shows that the auditory response and multimodal response still have their own distinct locations. Overall individual animal effect is significant in determining the location but *y* value is more sensitive to its impact than *x* value. The P-value of testing individual animal on *x* value is 0.1568, not a strong indication of id impact on *x*. (result from Table 5 and Table 6)

Table 5 MANOVA table of Control group data: Impact of two factors on *x* and *y*

	Source	*DF*	*Type III SS*	*Mean Square*	*F Value*	*P-value*
x	**color**	1	0.925766	0.925766	21.50	<0.0001
	id	8	0.515681	0.064460	1.50	0.1568
y	**color**	1	0.503584	0.503584	16.45	<0.0001
	id	8	1.832043	0.229005	7.48	<0.0001

Table 6 P-values of MANOVA on Control group: Testing overall factor effect

Statistic	*No Overall color Effect*	*No Overall id Effect*
Wilks' Lambda	0.0084	<0.0001
Pillai's Trace	0.0085	<0.0001
Hotelling-Lawley Trace	0.0084	<0.0001
Roy's Greatest Root	0.0024	<0.0001

2. Within the Small lesion group, factor color is not a significant regressor in determining the location of response points anymore based on P-values bigger than 0.05, while factor individual animal still plays significant role because of the very small P-values. Still *y* value is more sensitive to the two factors' impact than *x* value. This conclusion of factor individual animal shows that within Small lesion group, all the animals' reactions to the surgery are not consistent. (result from Table 7 and Table 8)

Table 7 MANOVA table of Small lesion group: Impact of two factors on *x* and *y*

	Source	*DF*	*Type III SS*	*Mean Square*	*F Value*	*P-value*
x	**color**	2	0.043011	0.021506	0.42	0.6568
	id	7	0.967475	0.138210	2.71	0.0099
y	**color**	2	0.214012	0.107006	2.92	0.0553
	id	7	0.835439	0.119348	3.26	0.0024

Table 8 P-values of MANOVA on Small lesion group: Testing overall factor effect

Statistic	*No Overall color Effect*	*No Overall id Effect*
Wilks' Lambda	0.1514	<0.0001
Pillai's Trace	0.1514	<0.0001
Hotelling-Lawley Trace	0.1517	<0.0001
Roy's Greatest Root	0.0520	<0.0001

3. Within the Large lesion group, factor color is not a significant factor anymore based on the much bigger P-values than 0.05. For the impact of factor individual animal, in general, it still plays significant role determining the location of response points because of the extremely small P-value. But when we check the impact of factor individual animal on x value and y value respectively, we found that factor individual animal has more influence on y value than on x value. But we have to be careful reaching this conclusion because we only have 3 animals to analyze in this group. Any potential forth animal's result might change the result dramatically. (result from Table 9 and Table 10)

Table 9 MANOVA table of Large lesion group: Impact of two factors on x and y

	Source	*DF*	*Type III SS*	*Mean Square*	*F Value*	*P-value*
x	**color**	2	0.220914	0.110457	1.38	0.2559
	id	2	0.273124	0.136562	1.71	0.1863
y	**color**	2	0.030241	0.015121	0.48	0.6211
	id	2	1.052622	0.526311	16.65	<0.0001

Table 10 P-values of MANOVA on Large lesion group: Testing overall factor effect

Statistic	*No Overall color Effect*	*No Overall id Effect*
Wilks' Lambda	0.4537	<0.0001
Pillai's Trace	0.4507	<0.0001
Hotelling-Lawley Trace	0.4549	<0.0001
Roy's Greatest Root	0.2244	<0.0001

We can see from the figures provided by the Biology Department that the blue and mixed color points in Control group's animals have their own locations---not much mixture of each other. But for animals in Small lesion and Large lesion group, it seems all colored points are mingled together, especially the horizontal location (x value). This phenomenon and conclusion are consistent with the conjecture from Dr. Sarah Pallas and Yuting Mao that after the damage to the midbrain, during the recovery process, the visual response points start to take over the primary auditory cortex, where the auditory response were at before the midbrain damage.

3.3 Permutation Test to Detect the Distribution Difference of Same Colored Points from Different Lesion Groups

Before we applied the Permutation Test combined with Kolmogorov-Smirnov Test and Wilcoxon Rank Sum Test to the real data, we carry a simulation study to investigate the power of the test near the estimated parameter values. Furthermore, we check the powers at the null hypothesis if they coincide with the significance levels. Hence, the simulation results reflect the goodness of the method applied to our data. We first create several animals' data for each distribution group assuming they have bi-variate normal distribution. After we standardize them into a Cartesian system, we applied the three steps introduced in Chapter two of this thesis. We find the power of the test under the null hypothesis situation when the two groups have same distribution. We also create the situations of increasing numbers of observation to see how good our method will be when the sample size increases. From Table 11, we can see the powers at null hypothesis match the significance level we pre-assigned. Just a median deviation of means or/and variance-covariance matrices, we obtain reasonably powers, which are better than we originally expected. Therefore, we are comfortable to use this method to test the equality of two-dimensional distributions. (Table 11)

Now we apply the test on real data. The following table is the P-value result of our Permutation Test on same colored points from different lesion groups. According to the result, we could only reject the null hypothesis that the blue points between Control group and Large lesion group have same distribution with strong evidence. For the other scenarios, we could not detect obvious different distribution patterns. It should be noticed that the comparison of red points between Small lesion group and Large lesion group gives us a 0.10 P-value. But if we

Table 11 Permutation Test simulation table

Power table																					
					Null center					Small deviation					Medium deviation						
				μ1= μ2=(0.15,0.5)				μ1=(0.15,0.5), μ2=(0.2,0.5)				μ1=(0.15,0.5), μ2=(0.2, 0.45)				μ1=(0.15,0.5), μ2=(0.15,0.4)				μ1=(0.15,0.5), μ2=(0.05,0.6)		
1-alpha	1-alpha			90%	95%	99%		90%	95%	99%		90%	95%	99%		90%	95%	99%		90%	95%	99%
n=8 k=3	points=10	both ∑s=(0.03 0.005,		0.101	0.051	0.008		0.180	0.105	0.026		0.198	0.106	0.023		0.353	0.228	0.104		0.496	0.345	0.138
56 ranks	points=20	0.005 0.03)		0.102	0.048	0.011		0.218	0.151	0.048		0.343	0.206	0.067		0.599	0.445	0.208		0.848	0.739	0.459
	points=30			0.105	0.043	0.008		0.319	0.218	0.087		0.437	0.279	0.108		0.792	0.665	0.399		0.957	0.894	0.706
	points=10	∑1=(0.03 0.005,		0.110	0.052	0.010		0.165	0.089	0.028		0.209	0.101	0.025		0.348	0.213	0.084		0.475	0.321	0.129
	points=20	0.005 0.03)		0.108	0.046	0.010		0.260	0.156	0.053		0.307	0.180	0.052		0.585	0.445	0.213		0.784	0.654	0.362
	points=30	∑2=(0.04 0.01,		0.135	0.064	0.010		0.334	0.206	0.076		0.484	0.340	0.112		0.774	0.658	0.383		0.938	0.860	0.625
		0.01 0.04)																				
	points=10	∑1=(0.03 0.005,		0.325	0.203	0.071		0.400	0.255	0.098		0.499	0.350	0.164		0.664	0.531	0.303		0.834	0.724	0.496
	points=20	0.005 0.03)		0.632	0.460	0.195		0.694	0.542	0.272		0.828	0.705	0.451		0.964	0.912	0.761		0.991	0.977	0.908
	points=30	∑2=(0.01 0.001,		0.828	0.709	0.411		0.873	0.775	0.516		0.965	0.905	0.717		0.998	0.992	0.953		1.000	1.000	0.990
		0.001 0.01)																				
n=10 k=4	points=10	both ∑s=(0.03 0.005,		0.100	0.056	0.012		0.190	0.114	0.027		0.262	0.169	0.060		0.416	0.293	0.138		0.639	0.505	0.292
210 ranks	points=20	0.005 0.03)		0.090	0.044	0.008		0.289	0.193	0.080		0.416	0.277	0.120		0.709	0.613	0.390		0.937	0.870	0.704
	points=30			0.116	0.051	0.008		0.370	0.260	0.120		0.576	0.444	0.222		0.890	0.820	0.644		0.993	0.975	0.909
	points=10	∑1=(0.03 0.005,		0.116	0.065	0.017		0.185	0.123	0.044		0.233	0.147	0.058		0.427	0.317	0.133		0.619	0.460	0.264
	points=20	0.005 0.03)		0.113	0.058	0.021		0.311	0.202	0.076		0.448	0.318	0.126		0.732	0.602	0.370		0.925	0.847	0.654
	points=30	∑2=(0.04 0.01,		0.135	0.084	0.024		0.431	0.295	0.134		0.596	0.455	0.229		0.877	0.777	0.568		0.991	0.973	0.889
		0.01 0.04)																				
	points=10	∑1=(0.03 0.005,		0.416	0.275	0.133		0.505	0.370	0.177		0.595	0.448	0.257		0.795	0.684	0.464		0.919	0.867	0.699
	points=20	0.005 0.03)		0.751	0.609	0.378		0.824	0.698	0.463		0.929	0.856	0.681		0.994	0.990	0.937		0.999	0.997	0.991
	points=30	∑2=(0.01 0.001,		0.939	0.885	0.693		0.957	0.916	0.775		0.991	0.981	0.938		1.000	1.000	0.998		1.000	1.000	0.998
		0.001 0.01)																				
n=11 k=5	points=10	both ∑s=(0.03 0.005,		0.099	0.053	0.007		0.212	0.140	0.038		0.295	0.190	0.059		0.456	0.337	0.155		0.735	0.625	0.332
462 ranks	points=20	0.005 0.03)		0.095	0.035	0.004		0.346	0.236	0.093		0.471	0.337	0.156		0.821	0.734	0.475		0.968	0.930	0.780
	points=30			0.102	0.047	0.007		0.472	0.356	0.155		0.668	0.515	0.251		0.941	0.892	0.718		0.996	0.992	0.957
	points=10	∑1=(0.03 0.005,		0.120	0.060	0.014		0.215	0.124	0.037		0.268	0.157	0.040		0.477	0.355	0.166		0.668	0.544	0.285
	points=20	0.005 0.03)		0.140	0.069	0.016		0.370	0.261	0.107		0.526	0.389	0.160		0.795	0.679	0.432		0.961	0.914	0.722
	points=30	∑2=(0.04 0.01,		0.179	0.105	0.026		0.520	0.382	0.164		0.710	0.580	0.305		0.921	0.864	0.667		0.997	0.989	0.925
		0.01 0.04)																				
	points=10	∑1=(0.03 0.005,		0.454	0.327	0.127		0.575	0.438	0.179		0.679	0.536	0.295		0.871	0.804	0.577		0.950	0.917	0.773
	points=20	0.005 0.03)		0.836	0.743	0.457		0.904	0.833	0.577		0.964	0.932	0.781		0.998	0.995	0.970		1.000	1.000	0.996
	points=30	∑2=(0.01 0.001,		0.970	0.929	0.768		0.983	0.964	0.842		1.000	0.997	0.964		1.000	1.000	0.998		1.000	1.000	1.000
		0.001 0.01)																				

check all the Small lesion group animals, we will find that there are total 8 animals but only 5 of them have shown red points. We used these 5 animals for the permutation test. Within these 5 animals, some animals only have 1 or 2 red point. Because of the scarcity and sparsity of the data, we should be cautious interpreting this comparison result.

Table 12 Permutation Test result (P-value table) of same color dots between lesion groups

color	*Control vs. Small*	*Control vs. Large*	*Small vs. Large*
Green	**0.8964**	**0.30**	**0.66**
blue	**0.2548**	**0.0091**	**0.1091**
red	**N/A**	**N/A**	**0.10**

CHAPTER FOUR: CONCLUSION AND FUTURE RESEARCH

In this thesis, we discussed the methodologies of three different tests. Since Permutation Test method has no available SAS code to use, we programmed that part. Before we applied it on the real data, we first tried it using simulation. Then we applied these methodologies on the data provided by Biology Department to give a complete analysis.

The result indicates that the factor lesion size and the factor color are closely associated. Within Control group, blue points (auditory responses) dominate the primary auditory cortex and there is no red points (visual responses) at all. When the midbrain is damaged (Small lesion and Large lesion), the animal's red points (visual response) start to take over some of the primary auditory cortex during the recovery process. This result confirms Dr. Sarah Pallas and Yuting Mao's theory about the recovery process of ABI patients. We also notice that when the lesion size increases from Small to Large, more visual responses appear in the primary auditory cortex.

In general, the location of each response point is determined by the individual animal, that point's color and what kind of lesion surgery that animal was given. But when we go into each lesion group and run the Multivariate Analysis of Variance Test, we found that within Small lesion and Large lesion group, the factor color is not a significant regressor anymore. Factor of individual animal plays important role in determining the location of that point. This is not a good biological result since it means each individual animal's performance is not consistent. This is especially obvious in Small lesion group. We could see five animals from Small lesion group have red points but other three animals have none. Maybe new experimental method can improve this problem in the future.

The Permutation Test shows us that only for the blue points (auditory responses) between Control and Large lesion group, we are certain that they have different distribution. Some animals' case brought difficulty to our analysis. For example, five out of eight animals in Small lesion group developed red points. Some of them only have one or two red points. We doubt that this scarcity of data would give us reliable conclusion.

Due to the experiment's special situation, we don't have a lot of data sets to work with. That might impair part of our result. We hope that we could be provided more data so to work our more reliable conclusions.

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Appendix A: SAS Code

1. Frequency Test Code

```
options nodate notes source print=on;


/*** read in normal animals' data *****/
proc import datafile='D:\research with Dr. Hsu\data\normal\F07-48only.xls'
                              out=normal48 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F07-157only.xls'
                              out=normal157 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F07-184only.xls'
                              out=normal184 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F07-243only.xls'
                              out=normal243 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F08-23only.xls'
                              out=normal23 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F08-55only.xls'
                              out=normal55 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\08-169.xls'
                              out=normal169 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\08178.xls'
                              out=normal178 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F08-145only.xls'
                              out=normal145 dbms=excel replace;
run;

/***** read in small leision animals' data  ******/
proc import datafile='D:\research with Dr. Hsu\data\small\F07-38only.xls'
                              out=small38 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\F07-52only.xls'
                              out=small52 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\F07-176only.xls'
                              out=small176 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\F08-09only.xls'
                              out=small09 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\0915.xls'
                              out=small15 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\08201.xls'
                              out=small201 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\08240.xls'
```

```
                              out=small240 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\08253.xls'
                              out=small253 dbms=excel replace;
run;


/***** read in large leision animals' data  *****/
proc import datafile='D:\research with Dr. Hsu\data\large\F07-61only.xls'
                              out=large61 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\large\F07-107only.xls'
                              out=large107 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\large\0921.xls'
                              out=large21 dbms=excel replace;
run;

data all;
      set normal48 normal157 normal184 normal243 normal23 normal55 normal169
normal178 normal145
                  small38 small52 small176 small09 small15 small201 small240
small253 large61 large107 large21;
run;


proc freq;
      tables lesion*color/CHISQ FISHER;
run;
```

2. Permutation test, simulation part

```
options nonotes nosource mprint=on;

/*****         START of all the macro components      ********/
%macro get_seeds(n);    /**** n is number of seeds you want ***/
      proc iml;
            x=J(&n,1,.);
            call randgen(x, 'UNIFORM');
            b=FLOOR(x*1000000000);

            create seeds from b;
            append from b;
            close seeds;
      quit;
%mend get_seeds;



%macro create_binormal(mu1, mu2, a, d, c, n, index);
proc iml;
      mu = {&mu1, &mu2};
      sigma = {&a &d, &d &c};
      series = J(&n, 2, 0);
      xy&index=J(&n, 2, 0);
```

```
      use seeds;
      read all into b;
      close seeds;;
      seed=b[&index];

      call vnormal (series, mu, sigma, &n, seed);

      do i=1 to &n;
            xy&index[i,1] =
series[i,1]/(1+sqrt(series[i,1]**2+series[i,2]**2)) ;
            xy&index[i,2] =
series[i,2]/(1+sqrt(series[i,1]**2+series[i,2]**2)) ;
      end;

      create animal&index from xy&index;
      append from xy&index;
      close animal&index;
quit;
%mend create_binormal;


/*Macro: get Comulative Distribution distance and then kolomogorov-smirnor
value for these two animals under matrix
   first is the name of the first data set, second is the name of the second
data set*/
%macro k(first, second);

      use &first;
      read all var{col1 col2}  into firstmat;
      close &first;

      use &second;
      read all var{col1 col2} into secmat;
      close &second;

      matcom=firstmat//secmat;
      a=nrow(firstmat);
      b=nrow(secmat);
      total=a+b;
      D= J(total,1, 0);

      do j=1 to total;
            m= 0;
            do i=1 to a;
                  if firstmat[i, ] <=  matcom[j, ]  then m=m+1;
            end;
            n=0;
            do i=1 to b;
                  if secmat[i, ] <=   matcom[j, ] then n=n+1;
            end;
            D[j,1] = abs(m/a-n/b);
      end;
      max_D=max(D);
      k=sqrt(a*b/(a+b))*max_D;
 %mend k;

%macro allk(totalnum);
```

```
      KStemp=J(&totalnum, &totalnum,0);
      R=J(&totalnum, &totalnum,0);
      %do i=1 %to %eval(&totalnum-1);
            %do j=%eval(&i+1)  %to &totalnum;
                  %k(animal&i, animal&j)
                  KStemp[&i,&j]=k;
                  KS=ROUND(KStemp,.00001);
            %end;
      %end;
%mend allk;


/* Macro: get all combinations of k out of n  */
%macro Wilcox_rank_list(k,n);
ods exclude Plan.Plan1.FInfo;
ods exclude Plan.Plan1.Plan;
ods output Plan=Combinations;
proc plan;
      factors Block=%sysfunc(comb(&n, &k)) ordered
              Treat= &k of &n comb;
run;
data Combinations;
      set Combinations;
      drop Block;
run;

proc iml;
            %allk(&n)
            num=&n;
            adjust=0.5*(num*num+num);
            R_temp=ranktie(KS)-adjust;

            do i=2 to num;
                  do j=1 to i-1;
                        R[i,j]=R_temp[j,i];
                  end;
            end;
            do i=1 to num-1;
                  do j=i+1 to num;
                        R[i,j]=R_temp[i,j];
                  end;
            end;

      use Combinations;
      read all into L;
      close Combinations;
      row_num=nrow(L);
      col_num=ncol(L);

      wr=J(row_num,1,0);
      Lvector=J(1,col_num,0);
      do s=1 to row_num;
            do j=1 to col_num;
                  Lvector[j]=L[s,j];
            end;

            do j=1 to col_num;
```

```
                do m=0 to col_num;
                      if m=0 then do i=1 to Lvector[1]-1;
                                                  wr[s]=wr[s]+R[i,
Lvector[j]];
                                                  end;
                      if m>0 & m<col_num then do i=Lvector[m]+1 to
Lvector[m+1]-1;

      wr[s]=wr[s]+R[i, Lvector[j]];
                                                                  end;
                      if m=col_num then do i=Lvector[m]+1 to &n;
                                                  wr[s]=wr[s]+R[i,
Lvector[j]];
                                                  end;
                end;
            end;
      end;

      create WRlist from wr;
      append from wr;
      close WRlist;
quit;
/* find 90% 95% 99% percentile of WR */
ods output Means.Summary=cutoff_point;
ods exclude Means.Summary;
proc means data=WRlist p90 p95 p99 max;
      var col1;
run;
%mend Wilcox_rank_list;



%macro get_percentage_point(k, n, num_points); /* how many times of
simulation is pre-set to be 1000 times  */
      %Simulation_koutofn_null(&k,&n, &num_points)
      data standards;   set cutoff_point; run;
      data WR1000; set WR_FIRST; run;
      %do index=1 %to 999;   /* I used i before but it didn't work. i is used
before,and not closed */
            %Simulation_koutofn_null(&k, &n, &num_points)
            data standards;   set standards cutoff_point; run;
            data WR1000; set WR1000 WR_FIRST; run;
      %end;
%mend get_percentage_point;



/* below is a macro to get a single rank for a group of k A's and (n-k) B's,
it is used for simulation for 1000 such times  */
%macro rankAandB(k,n);
proc iml;
            %allk(&n)
            num=&n;
            adjust=0.5*(num*num+num);
            R_temp=ranktie(KS)-adjust;

            do i=2 to num;
```

```
                do j=1 to i-1;
                      R[i,j]=R_temp[j,i];
                end;
          end;
          do i=1 to num-1;
                do j=i+1 to num;
                      R[i,j]=R_temp[i,j];
                end;
          end;

      wr=J(1,1,0);
      do j=1 to &k;
                do i=&k+1 to num;
                            wr=wr+R[i, j];
                end;
      end;

      create WR1 from wr;
      append from wr;
      close WR1;
quit;
%mend rankAandB;

%macro Simulation_koutofn_alt(k, n, Amu1, Amu2, Aa, Ad, Ac, Anum_points,
Bmu1, Bmu2, Ba, Bd, Bc, Bnum_points);
%get_seeds(&n)
%do repeatA=1 %to &k;
            %create_binormal(&Amu1,  &Amu2,  &Aa,  &Ad, &Ac,  &Anum_points,
&repeatA)
      %end;

%do repeatB=%eval(&k+1) %to &n;
            %create_binormal(&Bmu1,  &Bmu2,  &Ba,  &Bd,  &Bc,  &Bnum_points,
&repeatB)
      %end;

%rankAandB(&k, &n)
%mend Simulation_koutofn_alt;
/*****     END of all the macro components      ******/




/*****     START of simulation        *******/
/****************          3 out of 8 case         *********************/

/*do it 1000 times to get the comparing standards for power, need to change
number of points here,
after the comparing points are found, it is not used anymore*/
%macro Simulation_koutofn_null(k, n, num_points); /* mu and sigma is pre-set,
could change to other numbers */
%get_seeds(&n)
      %do repeat=1 %to &n;
            %create_binormal(0.05, 0.6, 0.0025, 0.0001, 0.0025, &num_points,
&repeat)
```

```
      %end;

      %Wilcox_rank_list(&k,&n)
%mend Simulation_koutofn_null;
proc printto log="NUL:";
run;
%get_percentage_point(3, 8, 30) /* numbers are k, n, number of points*/

proc means data=standards;
run;

/*****************************************************/
/*  simulation for powers with different dist'n of A and B, do it 1000 times
*/
/* find power for one pair of 3 As and 5 Bs, change the center and variance
matrix of two groups A and B here */
 /********     different for each specific mu, sigma case, need to change
numbers and macro ******/
%macro get_power;
      %Simulation_koutofn_alt(3, 8, 0.15, 0.5, 0.03, 0.005, 0.03, 10, 0.05,
0.6,  0.01, 0.001, 0.01, 10)
      data WR1000;
            set WR1;
      run;
      %do times=1 %to 999;
      %Simulation_koutofn_alt(3, 8, 0.15, 0.5, 0.03, 0.005, 0.03, 10, 0.05,
0.6,  0.01, 0.001, 0.01, 10)
      data WR1000;
            set WR1000 WR1;
      run;
      %end;
%mend get_power;

proc printto  LOG="NUL:";
run;
%get_power

data power;
      set WR1000;
      retain i 0 j 0 k 0;
            if COL1>243.9 then i=i+1;
            if COL1>253.8 then j=j+1;
            if COL1>270.6284 then k=k+1;
            power_90=i/1000; power_95=j/1000; power_99=k/1000;

run;

proc print data=power;
run;




/************************* 4 out 0f 10 case  *****************/
```

```
/*do it 1000 times to get the comparing standards for power, need to change
number of points here,
after the comparing points are found, it is not used anymore*/
%macro Simulation_koutofn_null(k, n, num_points); /* mu and sigma is pre-set,
could change to other numbers */
%get_seeds(&n)
      %do repeat=1 %to &n;
            %create_binormal(0.05, 0.6, 0.03, 0.005, 0.03, &num_points,
&repeat)
      %end;

      %Wilcox_rank_list(&k,&n)
%mend Simulation_koutofn_null;

proc printto log="NUL:";
run;
%get_percentage_point(4, 10, 30) /* numbers are k, n, number of points*/

proc means data=standards;
run;

/*****************************************************/
/*  simulation for powers with different dist'n of A and B, do it 1000 times
*/
/* find power for one pair of 3 As and 5 Bs, change the center and variance
matrix of two groups A and B here */
 /********     different for each specific mu, sigma case, need to change
numbers and macro ******/
%macro get_power;
      %Simulation_koutofn_alt(4, 10, 0.15, 0.5, 0.03, 0.005, 0.03, 10, 0.05,
0.6, 0.01, 0.001, 0.01 , 10)
      data WR1000;
            set WR1;
      run;
      %do times=1 %to 999;
      %Simulation_koutofn_alt(4, 10, 0.15, 0.5, 0.03, 0.005, 0.03, 10, 0.05,
0.6, 0.01, 0.001, 0.01 , 10)
      data WR1000;
            set WR1000 WR1;
      run;
      %end;
%mend get_power;

proc printto  LOG="NUL:";
run;
%get_power

data power;
      set WR1000;
      retain i 0 j 0 k 0;
            if COL1>603.0129 then i=i+1;
            if COL1>621.0318 then j=j+1;
            if COL1>653.3804 then k=k+1;
            power_90=i/1000; power_95=j/1000; power_99=k/1000;

run;
```

```
proc print data=power;
run;
```

```
/**********************            5 out of 11 case      ******************/
%macro Simulation_koutofn_null(k, n, num_points); /* mu and sigma is pre-set,
could change to other numbers */
%get_seeds(&n)
      %do repeat=1 %to &n;
            %create_binormal(0.05, 0.6, 0.0025, 0.0001, 0.0025, &num_points,
&repeat)
      %end;

      %Wilcox_rank_list(&k,&n)
%mend Simulation_koutofn_null;

proc printto log="NUL:";
run;
%get_percentage_point(5, 11, 30) /* numbers are k, n, number of points*/

proc means data=standards;
run;

/*****************************************************/
/*  simulation for powers with different dist'n of A and B, do it 1000 times
*/
/* find power for one pair of 3 As and 5 Bs, change the center and variance
matrix of two groups A and B here */
 /********     different for each specific mu, sigma case, need to change
numbers and macro ******/
%macro get_power;
      %Simulation_koutofn_alt(5, 11, 0.15, 0.5, 0.03, 0.005, 0.03, 10, 0.15,
0.5, 0.01, 0.001, 0.01 , 10)
      data WR1000;
            set WR1;
      run;
      %do times=1 %to 999;
      %Simulation_koutofn_alt(5, 11, 0.15, 0.5, 0.03, 0.005, 0.03, 10, 0.15,
0.5, 0.01, 0.001, 0.01 , 10)
      data WR1000;
            set WR1000 WR1;
      run;
      %end;
%mend get_power;

proc printto  LOG="NUL:";
run;
%get_power

data power;
      set WR1000;
```

```
      retain i 0 j 0 k 0;
            if COL1>903.3778 then i=i+1;
            if COL1>926.6501 then j=j+1;
            if COL1>975.7615 then k=k+1;
            power_90=i/1000; power_95=j/1000; power_99=k/1000;

run;

proc print data=power;
run;
```

3. Permutation test using real data to get conclusion

```
/*********         conclusion using real data: see if the dots' dist'n are
different between lesion groups         **********/
/*****      need to use %k, %allk and %Wilcox_rank_list (k,n)      ******/

options nodate notes source print=on;

/*** read in normal animals' data *****/
proc import datafile='D:\research with Dr. Hsu\data\normal\F07-48only.xls'
                              out=normal48 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F07-157only.xls'
                              out=normal157 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F07-184only.xls'
                              out=normal184 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F07-243only.xls'
                              out=normal243 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F08-23only.xls'
                              out=normal23 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F08-55only.xls'
                              out=normal55 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\08-169.xls'
                              out=normal169 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\08178.xls'
                              out=normal178 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F08-145only.xls'
                              out=normal145 dbms=excel replace;
run;

/***** read in small leision animals' data  ******/
proc import datafile='D:\research with Dr. Hsu\data\small\F07-38only.xls'
                              out=small38 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\F07-52only.xls'
                              out=small52 dbms=excel replace;
run;
```

```
proc import datafile='D:\research with Dr. Hsu\data\small\F07-176only.xls'
                              out=small176 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\F08-09only.xls'
                              out=small09 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\0915.xls'
                              out=small15 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\08201.xls'
                              out=small201 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\08240.xls'
                              out=small240 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\08253.xls'
                              out=small253 dbms=excel replace;
run;


/***** read in large leision animals' data  *****/
proc import datafile='D:\research with Dr. Hsu\data\large\F07-61only.xls'
                              out=large61 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\large\F07-107only.xls'
                              out=large107 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\large\0921.xls'
                              out=large21 dbms=excel replace;
run;

/*******       comparisons between large and small lesion group      *******/
/*****        green dots        ******/
data animal1; set large61; if color=0; keep x y; rename x=col1 y=col2; run;
data animal2; set large107; if color=0;  keep x y;rename x=col1 y=col2; run;
data animal3; set large21; if color=0; keep x y; rename x=col1 y=col2;run;

data animal4; set small38; if color=0; keep x y; rename x=col1 y=col2;run;
data animal5; set small52; if color=0; keep x y;rename x=col1 y=col2; run;
data animal6; set small176; if color=0; keep x y; rename x=col1 y=col2;run;
data animal7; set small09; if color=0; keep x y; rename x=col1 y=col2;run;
data animal8; set small15; if color=0; keep x y; rename x=col1 y=col2;run;
data animal9; set small201; if color=0; keep x y; rename x=col1 y=col2;run;
data animal10; set small240; if color=0; keep x y;rename x=col1 y=col2; run;
data animal11; set small253; if color=0; keep x y;rename x=col1 y=col2; run;

proc printto;
run;
%Wilcox_rank_list(3,11)

proc print data=WRlist (obs=1);
run;
/***** blue  dots  *****/
data animal1; set large61; if color=1; keep x y; rename x=col1 y=col2; run;
data animal2; set large107; if color=1;  keep x y;rename x=col1 y=col2; run;
data animal3; set large21; if color=1; keep x y; rename x=col1 y=col2;run;
```

```
data animal4; set small38; if color=1; keep x y; rename x=col1 y=col2;run;
data animal5; set small52; if color=1; keep x y;rename x=col1 y=col2; run;
data animal6; set small176; if color=1; keep x y; rename x=col1 y=col2;run;
data animal7; set small09; if color=1; keep x y; rename x=col1 y=col2;run;
data animal8; set small15; if color=1; keep x y; rename x=col1 y=col2;run;
data animal9; set small201; if color=1; keep x y; rename x=col1 y=col2;run;
data animal10; set small240; if color=1; keep x y;rename x=col1 y=col2; run;
data animal11; set small253; if color=1; keep x y;rename x=col1 y=col2; run;

proc printto;
run;
%Wilcox_rank_list(3,11)

proc print data=WRlist (obs=1);
run;

/*****   red  dots (small group only has
small09,small15,small176,small201,small52 usable     *****/
data animal1; set large61; if color=2; keep x y; rename x=col1 y=col2; run;
data animal2; set large107; if color=2;  keep x y;rename x=col1 y=col2; run;
data animal3; set large21; if color=2; keep x y; rename x=col1 y=col2;run;

data animal4; set small52; if color=2; keep x y;rename x=col1 y=col2; run;
data animal5; set small176; if color=2; keep x y; rename x=col1 y=col2;run;
data animal6; set small09; if color=2; keep x y; rename x=col1 y=col2;run;
data animal7; set small15; if color=2; keep x y; rename x=col1 y=col2;run;
data animal8; set small201; if color=2; keep x y; rename x=col1 y=col2;run;


proc printto;
run;
%Wilcox_rank_list(3,8)

proc print data=WRlist (obs=1);
run;



/*****      comparisons between large and normal lesion group          *****/
/*****        green dots        ******/
data animal1; set large61; if color=0; keep x y; rename x=col1 y=col2; run;
data animal2; set large107; if color=0;  keep x y;rename x=col1 y=col2; run;
data animal3; set large21; if color=0; keep x y; rename x=col1 y=col2;run;

data animal4; set normal48; if color=0; keep x y; rename x=col1 y=col2;run;
data animal5; set normal157; if color=0; keep x y;rename x=col1 y=col2; run;
data animal6; set normal184; if color=0; keep x y; rename x=col1 y=col2;run;
data animal7; set normal243; if color=0; keep x y; rename x=col1 y=col2;run;
data animal8; set normal23; if color=0; keep x y; rename x=col1 y=col2;run;
data animal9; set normal55; if color=0; keep x y; rename x=col1 y=col2;run;
data animal10; set normal169; if color=0; keep x y;rename x=col1 y=col2; run;
data animal11; set normal178; if color=0; keep x y;rename x=col1 y=col2; run;
data animal12; set normal145; if color=0; keep x y;rename x=col1 y=col2; run;

proc printto;
run;
%Wilcox_rank_list(3,12)
```

```
proc print data=WRlist (obs=1);
run;

/*****       blue  dots           *****/
data animal1; set large61; if color=1; keep x y; rename x=col1 y=col2; run;
data animal2; set large107; if color=1;  keep x y;rename x=col1 y=col2; run;
data animal3; set large21; if color=1; keep x y; rename x=col1 y=col2;run;

data animal4; set normal48; if color=1; keep x y; rename x=col1 y=col2;run;
data animal5; set normal157; if color=1; keep x y;rename x=col1 y=col2; run;
data animal6; set normal184; if color=1; keep x y; rename x=col1 y=col2;run;
data animal7; set normal243; if color=1; keep x y; rename x=col1 y=col2;run;
data animal8; set normal23; if color=1; keep x y; rename x=col1 y=col2;run;
data animal9; set normal55; if color=1; keep x y; rename x=col1 y=col2;run;
data animal10; set normal169; if color=1; keep x y;rename x=col1 y=col2; run;
data animal11; set normal178; if color=1; keep x y;rename x=col1 y=col2; run;
data animal12; set normal145; if color=1; keep x y;rename x=col1 y=col2; run;

proc printto;
run;
%Wilcox_rank_list(3,12)

proc print data=WRlist (obs=1);
run;



/*   comparison of same colored dots between normal & small lesion groups
*/
/******       green dots           *********/
data animal1; set small38; if color=0; keep x y; rename x=col1 y=col2;run;
data animal2; set small52; if color=0; keep x y;rename x=col1 y=col2; run;
data animal3; set small176; if color=0; keep x y; rename x=col1 y=col2;run;
data animal4; set small09; if color=0; keep x y; rename x=col1 y=col2;run;
data animal5; set small15; if color=0; keep x y; rename x=col1 y=col2;run;
data animal6; set small201; if color=0; keep x y; rename x=col1 y=col2;run;
data animal7; set small240; if color=0; keep x y;rename x=col1 y=col2; run;
data animal8; set small253; if color=0; keep x y;rename x=col1 y=col2; run;

data animal9; set normal48; if color=0; keep x y; rename x=col1 y=col2;run;
data animal10; set normal157; if color=0; keep x y;rename x=col1 y=col2; run;
data animal11; set normal184; if color=0; keep x y; rename x=col1 y=col2;run;
data animal12; set normal243; if color=0; keep x y; rename x=col1 y=col2;run;
data animal13; set normal23; if color=0; keep x y; rename x=col1 y=col2;run;
data animal14; set normal55; if color=0; keep x y; rename x=col1 y=col2;run;
data animal15; set normal169; if color=0; keep x y;rename x=col1 y=col2; run;
data animal16; set normal178; if color=0; keep x y;rename x=col1 y=col2; run;
data animal17; set normal145; if color=0; keep x y;rename x=col1 y=col2; run;

proc printto;
run;
%Wilcox_rank_list(8,17)

proc print data=WRlist (obs=1);
run;
```

```
/*****          blue dots        *******/
data animal1; set small38; if color=1; keep x y; rename x=col1 y=col2;run;
data animal2; set small52; if color=1; keep x y;rename x=col1 y=col2; run;
data animal3; set small176; if color=1; keep x y; rename x=col1 y=col2;run;
data animal4; set small09; if color=1; keep x y; rename x=col1 y=col2;run;
data animal5; set small15; if color=1; keep x y; rename x=col1 y=col2;run;
data animal6; set small201; if color=1; keep x y; rename x=col1 y=col2;run;
data animal7; set small240; if color=1; keep x y;rename x=col1 y=col2; run;
data animal8; set small253; if color=1; keep x y;rename x=col1 y=col2; run;

data animal9; set normal48; if color=1; keep x y; rename x=col1 y=col2;run;
data animal10; set normal157; if color=1; keep x y;rename x=col1 y=col2; run;
data animal11; set normal184; if color=1; keep x y; rename x=col1 y=col2;run;
data animal12; set normal243; if color=1; keep x y; rename x=col1 y=col2;run;
data animal13; set normal23; if color=1; keep x y; rename x=col1 y=col2;run;
data animal14; set normal55; if color=1; keep x y; rename x=col1 y=col2;run;
data animal15; set normal169; if color=1; keep x y;rename x=col1 y=col2; run;
data animal16; set normal178; if color=1; keep x y;rename x=col1 y=col2; run;
data animal17; set normal145; if color=1; keep x y;rename x=col1 y=col2; run;

proc printto;
run;
%Wilcox_rank_list(8,17)

proc print data=WRlist (obs=1);
run;
```

4. MANOVA Test

```
options nodate notes source print=on;


/*** read in normal animals' data *****/
proc import datafile='D:\research with Dr. Hsu\data\normal\F07-48only.xls'
                              out=normal48 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F07-157only.xls'
                              out=normal157 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F07-184only.xls'
                              out=normal184 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F07-243only.xls'
                              out=normal243 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F08-23only.xls'
                              out=normal23 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F08-55only.xls'
                              out=normal55 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\08-169.xls'
                              out=normal169 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\08178.xls'
```

```
                              out=normal178 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\normal\F08-145only.xls'
                              out=normal145 dbms=excel replace;
run;

/***** read in small leision animals' data  ******/
proc import datafile='D:\research with Dr. Hsu\data\small\F07-38only.xls'
                              out=small38 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\F07-52only.xls'
                              out=small52 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\F07-176only.xls'
                              out=small176 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\F08-09only.xls'
                              out=small09 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\0915.xls'
                              out=small15 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\08201.xls'
                              out=small201 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\08240.xls'
                              out=small240 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\small\08253.xls'
                              out=small253 dbms=excel replace;
run;


/***** read in large leision animals' data  *****/
proc import datafile='D:\research with Dr. Hsu\data\large\F07-61only.xls'
                              out=large61 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\large\F07-107only.xls'
                              out=large107 dbms=excel replace;
run;
proc import datafile='D:\research with Dr. Hsu\data\large\0921.xls'
                              out=large21 dbms=excel replace;
run;

data all;
      set normal48 normal157 normal184 normal243 normal23 normal55 normal169
   normal178 normal145 small38 small52 small176 small09 small15 small201
   small240 small253 large61 large107 large21;
run;


/*****        do the test with all the data together            *****/
proc glm;
      class lesion color id;
      model x y = lesion color id(lesion);
      manova h=_all_;
      means lesion color id(lesion);
```

```
run;


/***     do the test within each lesion group     ****/
proc glm;
      class lesion color id;
      model x y = lesion color id;
      by lesion notsorted;
      manova h=_all_;
      means lesion color id;
run;
```

Appendix B: SAS Output

1. Frequency Test Result

The FREQ Procedure

Table of lesion by color

lesion(lesion) color(color)

Frequency Percent Row Pct Col Pct	0	1	2	Total
large	24 3.10 20.17 14.29	44 5.68 36.97 8.04	51 6.58 42.86 85.00	119 15.35
normal	36 4.65 10.14 21.43	319 41.16 89.86 58.32	0 0.00 0.00 0.00	355 45.81
small	108 13.94 35.88 64.29	184 23.74 61.13 33.64	9 1.16 2.99 15.00	301 38.84
Total	168 21.68	547 70.58	60 7.74	775 100.00

Statistics for Table of lesion by color

Statistic	DF	Value	Prob
Chi-Square	4	317.2724	<.0001
Likelihood Ratio Chi-Square	4	252.3756	<.0001
Mantel-Haenszel Chi-Square	1	97.5760	<.0001
Phi Coefficient		0.6398	
Contingency Coefficient		0.5390	
Cramer's V		0.4524	

Fisher's Exact Test

Table Probability (P)	2.812E-58
Pr <= P	3.031E-53

Sample Size = 775

2. MANOVA test result

The GLM Procedure

Dependent Variable: x x

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lesion	2	0.25947762	0.12973881	2.46	0.0859
color	2	0.36347503	0.18173752	3.45	0.0323
id(lesion)	17	1.73084747	0.10181456	1.93	0.0131

Dependent Variable: y y

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lesion	2	1.83761457	0.91880729	27.33	<.0001
color	2	0.24688497	0.12344249	3.67	0.0259
id(lesion)	17	3.88071811	0.22827754	6.79	<.0001

MANOVA Test Criteria and F Approximations for the Hypothesis of No Overall lesion Effect

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.92498529	14.95	4	1504	<.0001
Pillai's Trace	0.07505752	14.68	4	1506	<.0001
Hotelling-Lawley Trace	0.08105199	15.23	4	901.36	<.0001
Roy's Greatest Root	0.08047692	30.30	2	753	<.0001

MANOVA Test Criteria and F Approximations for the Hypothesis of No Overall color Effect

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.98198332	3.43	4	1504	0.0084
Pillai's Trace	0.01805084	3.43	4	1506	0.0085
Hotelling-Lawley Trace	0.01831245	3.44	4	901.36	0.0084
Roy's Greatest Root	0.01615971	6.08	2	753	0.0024

MANOVA Test Criteria and F Approximations for the Hypothesis of No Overall id(lesion) Effect

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.82977889	4.33	34	1504	<.0001
Pillai's Trace	0.17554481	4.26	34	1506	<.0001
Hotelling-Lawley Trace	0.19872451	4.39	34	1349	<.0001
Roy's Greatest Root	0.15815895	7.01	17	753	<.0001

Within Each Lesion Group:

---------- lesion=normal ----------

Dependent Variable: x x

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lesion	0	0.00000000	.	.	.
color	1	0.92576596	0.92576596	21.50	<.0001
id(lesion)	8	0.51568137	0.06446017	1.50	0.1568

Dependent Variable: y y

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lesion	0	0.00000000	.	.	.
color	1	0.50358445	0.50358445	16.45	<.0001
id(lesion)	8	1.83204338	0.22900542	7.48	<.0001

MANOVA Test Criteria and Exact F Statistics for the Hypothesis of No Overall color Effect

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.88539822	22.26	2	344	<.0001

Pillai's Trace	0.11460178	22.26	2	344	<.0001
Hotelling-Lawley Trace	0.12943529	22.26	2	344	<.0001
Roy's Greatest Root	0.12943529	22.26	2	344	<.0001

MANOVA Test Criteria and F Approximations for the Hypothesis of No Overall id(lesion) Effect

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.81250185	4.70	16	688	<.0001
Pillai's Trace	0.19092751	4.55	16	690	<.0001
Hotelling-Lawley Trace	0.22654567	4.86	16	559.33	<.0001
Roy's Greatest Root	0.20606283	8.89	8	345	<.0001

---------- lesion=small ----------

Dependent Variable: x x

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lesion	0	0.00000000	.	.	.
color	2	0.04301116	0.02150558	0.42	0.6568
id(lesion)	7	0.96747492	0.13821070	2.71	0.0099

Dependent Variable: y y

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lesion	0	0.00000000	.	.	.
color	2	0.21401228	0.10700614	2.92	0.0553
id(lesion)	7	0.83543938	0.11934848	3.26	0.0024

MANOVA Test Criteria and F Approximations for the Hypothesis of No Overall color Effect

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.97713314	1.69	4	580	0.1514
Pillai's Trace	0.02292332	1.69	4	582	0.1514
Hotelling-Lawley Trace	0.02334420	1.69	4	346.96	0.1517
Roy's Greatest Root	0.02052960	2.99	2	291	0.0520

MANOVA Test Criteria and F Approximations for the Hypothesis of No Overall id(lesion) Effect

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.84827928	3.55	14	580	<.0001
Pillai's Trace	0.15360360	3.46	14	582	<.0001
Hotelling-Lawley Trace	0.17663740	3.65	14	460.65	<.0001
Roy's Greatest Root	0.16302179	6.78	7	291	<.0001

---------- lesion=large ----------

Dependent Variable: x x

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lesion	0	0.00000000	.	.	.
color	2	0.22091442	0.11045721	1.38	0.2559
id(lesion)	2	0.27312428	0.13656214	1.71	0.1863

Dependent Variable: y y

Source	DF	Type III SS	Mean Square	F Value	Pr > F
lesion	0	0.00000000	.	.	.
color	2	0.03024118	0.01512059	0.48	0.6211
id(lesion)	2	1.05262260	0.52631130	16.65	<.0001

MANOVA Test Criteria and F Approximations for the Hypothesis of No Overall color Effect

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.96825049	0.92	4	226	0.4537
Pillai's Trace	0.03190560	0.92	4	228	0.4507
Hotelling-Lawley Trace	0.03262939	0.92	4	134.57	0.4549
Roy's Greatest Root	0.02655966	1.51	2	114	0.2244

MANOVA Test Criteria and F Approximations for the Hypothesis of No Overall id(lesion) Effect

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.75318912	8.60	4	226	<.0001
Pillai's Trace	0.25088707	8.18	4	228	<.0001
Hotelling-Lawley Trace	0.32227589	9.08	4	134.57	<.0001
Roy's Greatest Root	0.30450299	17.36	2	114	<.0001

Permutation Test please see the result of Table 11 and Table 12